RELATIONS BETWEEN PRE- AND POST-ANAEROBIC OXYGEN CONSUMPTION AND OXYGEN TENSION IN SOME FRESH WATER SNAILS

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Aquatic snails cannot be considered as particularly well adapted to anaerobic life since, in the absence of oxygen, they eventually lose their motility and die. They can, however, tolerate anaerobiosis for longer or shorter periods, these periods varying with the external temperature and also with the species (Jatzenko, 1928; Alsterberg, 1930; von Brand, Baernstein and Mehlman, 1950). Furthermore, it has been shown that some of the end products resulting from the anaerobic carbohydrate breakdown accumulate in the tissues (Mehlman and von Brand, 1951).

Animals giving this general type of response to lack of oxygen usually show in a subsequent aerobic recovery period a temporarily increased rate of oxygen consumption, a phenomenon variously known as repayment of an oxygen debt, respiratory rebound, or respiratory overshoot. Very little is known about this phase of snail physiology, and the few data available are contradictory. Borden (1931) found a very marked respiratory rebound in *Planorbis corneus,* while Füsser and Krüger (1951) did not find in the post-anaerobic respiration of this species and that of *Lymnaea stagnalis* a significant increase over the pre-anaerobic level.

Particular attention is paid in the present paper to the question of the relation between oxygen tension and pre- and post-anaerobic rates of oxygen consumption. It has been shown in many other invertebrates that the post-anaerobic oxygen consumption is frequently more dependent on the tension than the pre-anaerobic one. It has been claimed on the one hand that this indicates the participation of a different enzyme mechanism during the recovery period (Harnisch, 1935, 1936, 1951), while on the other hand von Buddenbrock (1939) considers the phenomenon as a necessary consequence of the increased respiratory level which would change the critical point at which diffusion ceases to furnish the organisms all the required oxygen. It has been pointed out previously (von Brand, 1947) that an extensive investigation on some non-parasitic invertebrate would be necessary to clarify the situation.

Materials and Methods

The following species of snails were used:

Planorbidae: *Australorbis glabratus,* laboratory-reared from Venezuelan stock, and *Helisoma duryi,* collected at Kenilworth, Maryland, but maintained in labora-

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tory aquaria for at least a week before being used. The Planorbidae employed ranged in weight from about 150 to 350 mg.

Physidae: Laboratory-reared *Aplexa nitens* from Texan stock, weighing from 200 to 500 mg.

Lymnaeidae: *Lymnaea stagnalis,* laboratory-reared, colony derived from laboratory-reared specimens obtained from Dr. L. E. Noland, University of Wisconsin. Juveniles weighing from about 100 to 600 mg. were used.

All respiration experiments were done by means of the conventional Warburg technique; vessels of about 16 ml. capacity were used, and 2 ml. of dechlorinated tap-water served as medium. Each specimen was used only for one experiment. The determinations were done at 30, 20 and 10° C. In general, a single snail was placed in a Warburg flask, but at 10° C. two snails frequently were used in order to obtain manometric readings of sufficient magnitude.

The usual experimental procedure was as follows: The rate of oxygen consumption was first established for 2.5 to 4 hours under atmospheric air (21 per cent oxygen). The gas phase was then changed, if required, by flushing the manometers with 100 per cent oxygen, 11 or 5 per cent oxygen in nitrogen (gases taken from steel cylinders; composition verified by analysis) and the respiratory rate again determined during 3 to 4 hours. Anaerobiosis was then established by flushing the manometers for 15 to 25 minutes with pure Linde nitrogen (99.99 per cent, further purified by passing over heated copper, pressure difference in manometer ca. 100 mm. Brodie fluid), and anaerobic conditions were maintained for 16 ± 0.5 hours. Shaking of the manometers was suspended after anaerobiosis was established since during this period no readings were taken. At the end of the 16 hours the vessels were removed from the manometers, the medium was withdrawn by means of a Pasteur pipette and replaced by fresh medium. In this way the excreted end-products were eliminated. They could otherwise have been reabsorbed or served as substrate for bacterial development during the recovery period. The manometers were then returned to the water bath and flushed with the same gas which had been used immediately preceding the anaerobic period. This flushing period served at the same time as temperature re-equilibration period. Readings were then taken usually for 8 hours, but in a few cases only for 7 or 7.5 hours.

Both pre- and post-anaerobic readings were taken at 15-minute intervals, but in experiments with *Australorbis glabratus* and *Helisoma duryi* at 10° C. the interval was 30 minutes. The data for each reading interval of each experiment were calculated separately. They were averaged and graphed for a given series. Owing to the many readings available for each experimental period, the respiratory pattern could be established with considerable accuracy. The experimental procedure permitted the direct comparison of the post-anaerobic oxygen consumption with the pre-anaerobic rate in both 21 per cent oxygen and in the same gas that was used immediately preceding the anaerobic period. In a few experiments, the pre-anaerobic relation between oxygen consumption in 21 per cent oxygen and the other tensions was established separately. Where in such cases post-anaerobic determinations were done, different snails were used and only one of the four gas mixtures was used both pre- and post-anaerobically.

TABLE I

Influence of oxygen tension on the pre- and post-anaerobic oxygen consumption of well-fed snails

Species	Temp. °C.	No. of expts.	Pre-anaerobic rates			Post-anaerobic maximal rate	
			Rate in 21 per cent O_2 mm.$^3O_2$/gm./hr.	O_2 per cent in exp. gas	Rate in exp. gas in per cent of rate in 21 per cent O_2	In per cent of pre-anaerobic rate in 21 per cent O_2	In per cent of pre-anaerobic rate in exp. gas
Australorbis glabratus	30	17	119.0±6.4	100	109±5.0	170±6.3	157±7.0
Australorbis glabratus	30	16	120.9±5.2	21	100	162±10.3	162±10.3
Australorbis glabratus	30	14	148.7±8.8	11	104±5.7	147±7.8	141±7.5
Australorbis glabratus	30	31	144.8±6.4	5	66±4.6	89±3.7	135±5.6
Australorbis glabratus	20	12	50.2±2.9	100	114±6.4	151±9.9	133±5.0
Australorbis glabratus	20	18	69.2±2.8	21	100	122±4.7	122±4.7
Australorbis glabratus	20	12	56.0±6.0	11	86±9.6	142±13.3	173±13.1
Australorbis glabratus	20	11	62.7±5.0	5	69±6.8	80±7.7	122±13.9
Australorbis glabratus	10	20	18.8±0.6	100	98±3.9	148±6.2	154±9.8
Australorbis glabratus	10	18	11.1±0.4	21	100	130±7.4	130±7.4
Australorbis glabratus	10	22	18.6±1.1	11	77±2.4	134±6.7	178±11.2
Australorbis glabratus	10	20	17.4±0.6	5	66±3.9	107±7.2	170±18.3
*Australorbis glabratus**	20	14	52.0±3.7**	100	114**	189**	170±6.9
*Australorbis glabratus**	20	17	58.6±4.8	21	100	145±8.0	145±8.0
*Australorbis glabratus**	20	13	72.7±3.8**	11	86**	120**	143±10.7
*Australorbis glabratus**	20	21	59.3±2.4**	5	69**	94**	142±7.3
Helisoma duryi	30	15	125.9±13.2	100	111±6.2	211±13.6	192±12.4
Helisoma duryi	30	16	137.3±3.4	21	100	156±6.0	156±6.0
Helisoma duryi	30	14	185.9±10.5	11	81±4.1	138±7.2	170±8.9
Helisoma duryi	30	14	181.8±4.7	5	50±4.0	72±3.3	152±6.6
Helisoma duryi	20	14	56.2±3.6	100	93±4.3	141±9.5	154±9.5
Helisoma duryi	20	13	49.2±5.2	21	100	166±10.0	166±10.0
Helisoma duryi	20	13	49.3±3.4	11	83±3.0	132±12.4	157±8.3
Helisoma duryi	20	12	45.6±4.6	5	64±3.0	112±8.3	179±13.7
Helisoma duryi	10	16	15.5±1.0	100	87±6.9	197±16.0	232±16.6
Helisoma duryi	10	16	13.7±0.7	21	100	148±9.5	148±9.5
Helisoma duryi	10	16	13.2±1.0	11	93±5.9	138±13.0	155±13.4
Helisoma duryi	10	16	15.0±0.4	5	79±3.6	168±18.3	217±18.3
Aplexa nitens	20	12	50.3±4.2	100	126±6.6	175±13.3	139±9.7
Aplexa nitens	20	12	59.9±7.0	21	100	154±11.6	154±11.6
Aplexa nitens	20	16	62.6±1.7	11	85±7.5	133±4.5	156±5.7
Aplexa nitens	20	9	42.1±4.5	5	74±9.9	144±23.1	186±31.6
Lymnaea stagnalis	20	14	150±7.6	100	93±4.7	128±6.0	139±7.1
Lymnaea stagnalis	20	15	84.9±9.1	21	100	115±4.6	115±4.6
Lymnaea stagnalis	20	16	149.6±12.3	11	73±3.2	69±6.6	95±8.1
Lymnaea stagnalis	20	14	109.5±9.4	5	52±2.8	54±5.8	105±10.0
Lymnaea stagnalis	10	17	41.8±1.3	100	96±3.2	117±1.1	124±6.2
Lymnaea stagnalis	10	17	35.4±1.3	21	100	122±9.2	122±9.2
Lymnaea stagnalis	10	16	35.3±2.0	11	91±4.8	106±5.3	116±3.6
Lymnaea stagnalis	10	17	34.2±2.3	5	60±3.5	74±5.0	126±6.8

The oxygen determinations were done at the same temperature used for the anaerobic exposure in all series, except those marked with an asterisk. In these the oxygen determinations were carried out at 20° C., but the snails were exposed to anaerobiosis at 30° C. In this series only the experimental gas was used pre-anaerobically; the values marked by two asterisks are calculated on the assumption that the pre-anaerobic oxygen consumption showed the same dependency on the tension as shown by the corresponding series done with the same species at the same temperature and shown elsewhere in this table. All averages are arithmetic averages and hence show a slight upward bias. The figures after the ± signs are the standard error of the mean.

Results

1. Oxygen tension and pre-anaerobic oxygen consumption

A study of the pre-anaerobic rates of oxygen consumption of *Australorbis glabratus* (Tables I and II) shows that in this species the percentage decline in oxygen consumption at tensions below 21 per cent oxygen was on the whole remarkably similar regardless of whether the metabolic rate was changed by using different temperatures or snails of different nutritional state. One curious observation was that the oxygen consumption remained virtually unaffected by lowering the oxygen concentration from 21 to 11 per cent at 30° C., while the rate was definitely lowered under analogous conditions at 20° and 10° C. It is not believed that this somewhat paradoxical finding is to be attributed to experimental errors because in previous experiments (von Brand, Nolan and Mann, 1948) a similar, or even somewhat more pronounced independence of the oxygen consumption on the tension had been found at 30° C.

Table II

Influence of starvation on the dependency of the pre-anaerobic oxygen consumption on the oxygen tension. The rates are expressed in mm.³ oxygen per gm. fresh weight per hour

Species	Temp °C.	No. exp.	Starvation days	Oxygen consumption in per cent oxygen		5 per cent rate in per cent of 21 per cent rate
				21	5	
Australorbis glabratus	30	31	0	144.8±6.4	95.7±4.2	66±4.6
Australorbis glabratus	30	17	14	64.1±1.4	46.7±2.5	73±3.7
Australorbis glabratus	20	11	0	62.7±5.0	41.3±3.6	69±6.8
Australorbis glabratus	20	23	14	34.2±1.3	22.7±1.8	66±4.2
Helisoma duryi	30	14	0	181.8±4.5	90.9±4.7	50±4.0
Helisoma duryi	30	12	14	55.6±6.3	42.4±4.0	78±2.5
Helisoma duryi	20	12	0	45.6±4.6	27.9±2.3	64±3.0
Helisoma duryi	20	10	14	29.4±1.9	20.4±1.6	73±7.1

In *Helisoma duryi* and *Lymnaea stagnalis,* it is evident that lowering the metabolic rate did lead to a reduction in the dependency of the respiratory rate on the tension, although the effect was not very pronounced.

In view of these variations it is difficult, if not impossible, to grade the snail species used as to the magnitude of influence of oxygen tension. Apparently only minor differences exist in this respect between the four species investigated and, within the range of tensions tested, the species with hemoglobin in their blood (*Australorbis* and *Helisoma*) are, at a given temperature, capable of securing only a slightly higher percentage of their maximal oxygen needs than the snails having hemocyanin (*Lymnaea* and *Aplexa*). This is in approximate agreement with the findings of Füsser and Krüger (1951) on *Planorbis* and *Lymnaea.* We are also in agreement with these workers as to the finding that the actual respiratory rate, at equal temperature, is higher in *Lymnaea* than the other species. Evidently, the temperature relationships of various snail species must vary, since in previous experiments no such difference was found at 30° C. (von Brand, Nolan and Mann, 1948).

2. Post-anaerobic respiration

All four snail species studied showed a marked respiratory rebound after having been exposed to 16 hours of anaerobiosis (Table I). In all cases the period of excess oxygen consumption lasted a long time and three different types of recovery curves could be distinguished. In type 1, characteristic for *Helisoma,* the post-anaerobic rate was higher than the pre-anaerobic level immediately after restoration of aerobic conditions (excluding, of course, the time necessary for changing the medium and the other necessary manipulations, for which no data are available). The rate remained at a high level for several hours and then began to approach the normal without, however, reaching it completely within the time of observation. In type 2, occurring frequently in *Australorbis* and *Lymnaea,* the oxygen consumption was increased from the beginning and remained at this same level for the entire period of 7 to 8 hours. In type 3, observed in some series of all snails and the only one seen in *Aplexa,* the post-anaerobic rate was initially relatively low. After a period, varying from about one to three hours, a transition to maximal respiratory activity (higher than pre-anaerobic) occurred and this rate was usually sustained for the balance of the observation period. In a few cases, especially with *Australorbis,* a distinct lowering of the rate occurred during the 7th or 8th hour. During the initial period, the oxygen consumption was either somewhat lower or higher than the pre-anaerobic rate, but in most instances it was surprisingly close to the pre-anaerobic level, giving, perhaps erroneously, the impression that the normal (pre-anaerobic) metabolism was resumed immediately after restoration of aerobiosis, but that a certain induction period was necessary in these cases to initiate the actual respiratory rebound.

It is evident that owing to the long periods of "repayment" no definite data can be given concerning the total amount of oxygen repaid. However, in those cases where, after an initial high level, the post-anaerobic rate later approached the pre-anaerobic rate, it can be stated with some certainty, on the basis of a few observations made after 24 hours, that the repayment was far from complete. This is in contrast to the findings of Borden (1931) on *Planorbis corneus.* Borden used only very short periods of anaerobiosis; we used long periods. It is very probable that the proportion between anaerobic end-products accumulating in the tissues and being excreted varies under such different conditions and that this factor may be responsible for the differences in results. In our opinion the significance of the term "complete repayment of an oxygen debt" is sometimes over-emphasized. Insofar as invertebrates are concerned it does not imply anything of fundamental importance. The total amount of oxygen repaid will depend primarily upon the level of anaerobic metabolism, the nature of the anaerobic end-products, and the question to what extent they are excreted or stored in the tissues. None of these factors has necessarily a close quantitative connection with the pre-anaerobic oxidative metabolism, that is, with the amount of oxygen missed during anaerobiosis, but they will decide whether the post-anaerobic overshoot leads to an incomplete or complete repayment of the oxygen debt, or even to a more or less marked overpayment (review of the pertinent literature in von Brand, 1946).

Special attention was given to the relationship existing between pre-anaerobic rate of oxygen consumption and maximal post-anaerobic rate sustained over at

least two hours and usually over a much longer period. A study of Table I shows that in most series the post-anaerobic respiration was more dependent on the oxygen tension than the pre-anaerobic one if the pre-anaerobic consumption at 21 per cent oxygen is taken as reference point for both. It is also evident that

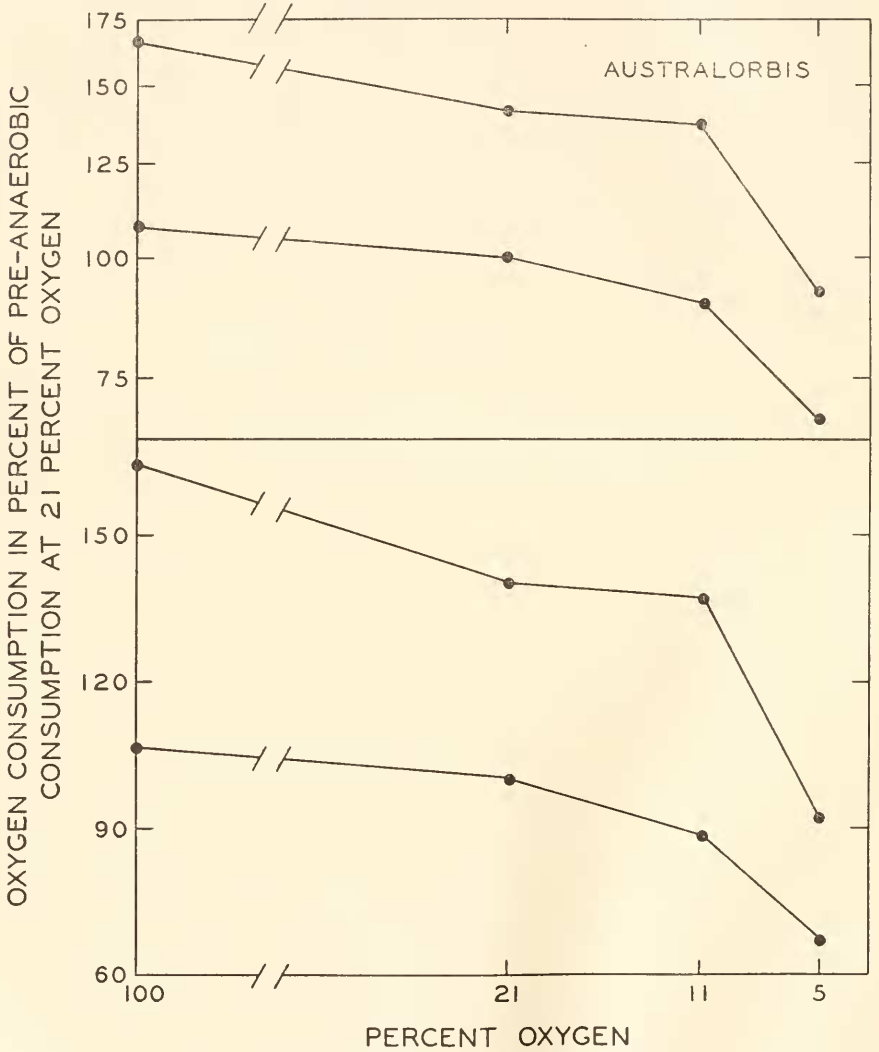

FIGURE 1. Dependency of the pre- and post-anaerobic respiration of *Australorbis glabratus* on the oxygen tension. Average values of all series plotted on an arithmetic scale (lower part of figure) and logarithmic scale (upper part of figure).

changes in the metabolic level induced by temperature changes have no decisive influence on the relation between pre- and post-anaerobic rates of oxygen consumption. The variations between the various series conducted with one and the same

snail species are fairly large, but this will surprise no one who has had experience with metabolic studies on this group of organisms.

Undoubtedly, some differences exist in the pre- to post-anaerobic ratios for the various conditions considered which appear significant from a purely statistical standpoint. By and large, however, the ratios are in the same region at all temperatures studied. Moreover, no constant pattern of temperature differences could be found when different oxygen tensions were used. Presumably, the significant differences reflect differences in the various batches of snails rather than differences due to temperature or oxygen tension. Separate batches of snails had to be used for each series and the entire experiments spread out over about a year.

As mentioned in a previous section the percentage decline in pre-anaerobic respiration at lowered oxygen tensions is unaffected by temperature changes only in *Australorbis*. It appears, therefore, permissible only in this species to average all the available series in order to minimize the biological variations by using the greatest possible number of observations. The results are shown in Figure 1. When plotted on an arithmetic scale, as has been done heretofore by all investigators, the greater dependency of the post-anaerobic oxygen consumption on the oxygen tension is quite evident. If considered from this standpoint alone, similar findings of Harnisch (1935, 1936) on insects and worms are apparently confirmed. If, however, the data are plotted on a logarithmic scale, a different picture emerges. The lines for the pre- and post-anaerobic respiration now rather closely parallel each other, indicating that the percentage increase of the post-anaerobic respiration over the pre-anaerobic rate is approximately identical at all tensions studied. The deviations of the two lines upon plotting on an arithmetic scale are quite apparently attributable to rate differences sustained by the pre-anaerobic respiration at the various oxygen tensions. Therefore, they actually do not constitute a proof for the contention that the post-anaerobic respiration is more dependent on the tension; rather, they prove the contrary.

The situation is essentially the same in the other snail species investigated. Again, considerable differences within series exist (Table I). The trend of the figure, however, is unmistakable. In all species, the post-anaerobic oxygen consumption is raised over the pre-anaerobic level to about the same degree regardless of the oxygen tension. A survey of Table I shows that *Helisoma* raised its post-anaerobic level highest, that of *Lymnaea* remained always relatively low, and the two other species were intermediate.

Discussion

The present investigation has shown that the oxygen consumption of four species of pulmonate snails is somewhat dependent on the tension below 21 per cent oxygen and that this dependency shows little connection with the actual rate of oxygen consumption. In *Australorbis glabratus* held in 5 per cent oxygen, the percentage decline (around 34 per cent) was the same whether (1) the actual respiratory level in 21 per cent oxygen was reduced from 145 $mm.^3$/gm./hr. to 17 $mm.^3$ by changing the temperature from 30° C. to 10° C., or (2) from 145 $mm.^3$ to 64 $mm.^3$ by starvation. In *Lymnaea stagnalis* and *Helisoma duryi*, the dependency was not quite as unalterable. In the latter species, for instance, the percentage decline in 5 per cent oxygen was 50 per cent when the metabolic level

was 182 mm.^{3}/gm./hr. in 21 per cent oxygen, 36 per cent at a level of 46 mm.^{3} and 21 per cent at a level of 15 mm.^{3}. It is evident that even in this latter case the oxygen consumption does not entirely conform to what would be expected if diffusion were the only limiting factor. According to the diffusion hypothesis, which at present seems to be accepted almost universally, lowering the oxygen tension below a critical point leads to a lowering of the over-all consumption because at lower tensions all the oxygen entering the body by diffusion is consumed by the superficial tissue layers and an internal layer becomes anoxic. Since temperature has but little influence on the rate of diffusion and starvation probably has none,[3] it would appear that the oxygen consumption of the above snails held in 5 per cent oxygen at 10° C. should have been about 100 per cent of that shown in air if the diffusion hypothesis held strictly. This seems to follow from the observation that at 30° C. in 5 per cent oxygen diffusion was able to bring much more oxygen into the body than the snails consumed at 10° C. in 21 per cent oxygen.

The above argument is not quite straightforward, however, because the snails have a circulatory system. It is conceivable that the oxygen transport within the body and/or the influence of temperature on the loading and unloading tension of the respiratory pigments may be the limiting factors. In order to shed some light on this point, several observations on the rate of heart-beat were done. In 15 *Australorbis* it was reduced at 10° C. to 13 per cent of the 30° rate, while the rate of oxygen consumption was reduced to 11 per cent. Similar experiments with 6 *Lymnaea* showed at 10° C. reductions in both rates corresponding to 44 and 43 per cent, respectively, of the 20° C. rate. This close parallelism between the rates of heart-beat and oxygen consumption tends therefore to support the above view. However, no such close parallelism was found in respect to the influence of starvation. A starvation period of two weeks reduced the rates of heart-beat and oxygen consumption of 15 *Australorbis* to 75 and 44 per cent of the pre-starvation rates. It seems therefore that another hitherto neglected factor may be of greater importance than believed in recent years: namely, the oxygen tension as such. It is of interest in this connection to note that Füsser and Krüger (1951) concluded from their study of normal and carbon monoxide-poisoned *Planorbis corneus* and *Lymnaea stagnalis* that the diffusion hypothesis alone does not suffice to explain the oxygen consumption-oxygen tension relationships. They assume a direct influence of the oxygen concentration but do not elaborate the possibility in greater detail. We shall return to this point after having discussed the post-anaerobic respiration of our objects.

All four species of snails used in the present study showed a respiratory rebound and in all species it was characterized by its long duration. In this respect the snails resembled the clam *Sphaerium corneum* (Jatzenko, 1928). In some other invertebrates, such as insects and worms, the repayment period lasts a shorter time, but the percentage increase of the post-anaerobic respiration may be appreciably higher (Harnisch, 1936, 1948; von Brand, 1947).

The post-anaerobic oxygen consumption of the snails appeared more dependent

[3] It is realized that this statement may need revision if further evidence becomes available. If starvation reduces the enzyme content of the cells materially, or if one accepts the free-radical mechanism for enzymatic reactions, starvation may change diffusion.

on the tension than the pre-anaerobic consumption when expressed in absolute terms or in per cent of the pre-anaerobic consumption in 21 per cent oxygen. In this respect the present findings parallel those of Harnisch (1936), but they do not necessarily support his conclusions. In his opinion the above phenomenon indicates that normal and excess respiration are mediated through different enzyme systems. However, our data show that in aquatic snails the post-anaerobic oxygen consumption is raised over the pre-anaerobic rate by approximately the same percentage, regardless of the tension employed. This apparently hitherto unrecognized relationship leads inevitably to the picture of greater dependency of the rebound respiration on the tension than that shown by the normal respiration, if expressed in absolute terms or in per cent of the pre-anaerobic value shown in 21 per cent oxygen. It is simply a consequence of the fact that increasing a graded series of figures by the same percentage makes the difference between elevated and base figure the greater, the greater the magnitude of the base figure is. Evidently, then, the oxygen tension/oxygen consumption relationship does not give any information as to whether different enzyme systems are responsible for the pre- and post-anaerobic oxygen consumption.

Our data also obviously contradict von Buddenbrock's (1939) view that the greater dependency of the overshoot respiration is attributable to a shift in the critical point towards a higher oxygen tension owing to the increased rate shown in this period. However, our observations do raise some new questions which at present can be answered only tentatively.

The obvious first question is why the post-anaerobic respiration should be raised by the same percentage over a wide range of tensions. Apparently, the degree of anaerobic stress plays only a minor role. The percentage increase was, within admittedly rather wide limits of variations, similar regardless of the temperature at which the specimens of one species had been exposed for an equal period to anaerobiosis. There is general agreement that the overshoot phenomenon is due to the accumulation of end-products of anaerobic metabolism. The increase in substrates would lead to an increased probability of enzyme and substrate molecules colliding (Zimmerman, 1949). The long period of repayment seems to indicate that a regulatory mechanism exists in snails which releases the "abnormal" substrates only slowly to the oxidative processes. This process might be geared to the rate of oxidations involving the normal substrates. Such an assumption would explain the phenomena observed.

Another very interesting question is the explanation of the physiological mechanism by which snails and other organisms are able to raise their post-anaerobic respiratory rate at those tensions where the normal respiration declines. This quite common phenomenon seems, surprisingly enough, never to have been discussed. It is a curious phenomenon because it indicates a regulatory mechanism that comes into play only post-anaerobically but not when the snail is exposed pre-anaerobically to oxygen concentrations allowing only 50 to 60 per cent of the normal maximal rate of respiration. If diffusion of oxygen into the body were the only limiting factor pre-anaerobically, one would obviously have to look for factors that would facilitate a greater influx of oxygen post-anaerobically. Such factors may be an increased surface by maximal extension of the foot, a facilitated oxygen release by the blood due to a Bohr effect, a steeper oxygen gradient in the

tissue layers adjacent to the diffusion surfaces owing to the accumulation of substrates, or due to an increased enzyme concentration. Such factors might well be important and possibly suffice to explain the phenomenon. Unfortunately, a highly organized animal, such as a snail, represents so complex a system that a complete analysis of these factors, experimentally or mathematically, would appear to be extremely difficult, if not impossible. It seems therefore appropriate to point out that at least one other alternative exists.

It is, for example, possible to discuss the present findings from a standpoint already touched upon above, namely, to raise the question whether the importance of the oxygen tension as such may not be greater than is usually suspected. Assuming that at least one enzymatic key-process of the cellular oxidation mechanism operates more efficiently at higher than at lower oxygen tensions, the following picture would appear. The relative inefficiency of a changed metabolic level in influencing the critical point of the pre-anaerobic oxygen consumption/oxygen tension relationship would be understandable without any auxiliary hypothesis since the influence of the tension would remain the same regardless of the temperature employed or the nutritional state of the snails. A necessary implication of this view would be that even at low tensions (low within limits, of course) the decrease of the pre-anaerobic oxygen consumption would not be due to the establishment of an anoxic zone in the deeper tissue layers. Even these tissues, like all the others, would get enough oxygen to allow a certain sub-optimal oxidative activity, the actual level of which would be determined on the one hand by the intracellular oxygen tension as influenced by the external tension, and on the other hand, by the availability of substrates. It may be pointed out in this connection that the fluoroacetate inhibition of citrate utilization in pigeon breast muscle is profoundly influenced by the oxygen tension (Massey and Rogers, 1951). Furthermore, Lehmann (1935) has found that succinic dehydrogenase was most active at oxygen tensions of 44 to 56 mm. Hg, while the activity significantly decreased at lower tensions. Finally, Schade and Levy (1949) have found in potato tissues, besides cytochrome oxidase, a terminal oxidase which was markedly sensitive to lowered oxygen tensions.

Insofar as post-anaerobic conditions are concerned, the increase in substrates and their gradual release to the oxidative processes would allow an increased oxygen consumption of all tissues and hence lead to an increase in the over-all oxygen consumption at all tensions where the above requirement is fulfilled, namely, that diffusion limitations would not be so severe as to lead to the establishment of an anoxic zone. The mechanism of the post-anaerobic increase in rate would hence be reduced to the accumulation of anaerobic metabolic end-products. The occurrence of the same percentage increase in post-anaerobic respiration over a wide range of tensions could also be understood better because it can be visualized that under otherwise identical conditions, the increased substrates and their gradual release may have, percentage wise, the same stimulatory effect on optimal and sub-optimal oxidative activity.

It should be understood that the above explanation is proposed only as a possible working hypothesis. It is realized that the available evidence does not constitute a definite proof. Furthermore, the above hypothesis is not mutually exclusive with the diffusion hypothesis. In any animal diffusion must become the

limiting factor if the tension is lowered sufficiently. The critical tension will vary from species to species, probably being highest in bulky animals lacking a circulatory system, like the actinians. Conversely, it is indisputable that somewhere along the line of oxygen tensions a critical point must be reached for any oxidation process below which it cannot function with full effectiveness. Insofar as the snail species studied are concerned, it is probable that both components enter the picture. Least affected by diffusion difficulties and hence most subject to the direct influence of tension seems to be *Australorbis glabratus,* while in *Helisoma duryi* and *Lymnaea stagnalis* diffusion difficulties seem to play a greater role as evidenced by the influence of the metabolic level on the critical point.

Summary

1. The respiration of four species of fresh water snails was somewhat dependent on the oxygen tension below 21 per cent oxygen with only minor differences among the various species.

2. The degree of dependency was influenced little, if any, by alteration of the metabolic rate of *Australorbis glabratus,* but some changes were obtained in the cases of *Helisoma duryi* and *Lymnaea stagnalis.*

3. All species showed a long lasting respiratory rebound after 16 hours anaerobiosis. The post-anaerobic respiration was more dependent on the oxygen tension than the pre-anaerobic respiration if referred to the normal rate shown at 21 per cent oxygen. But if the post-anaerobic rates were compared with the rates sustained pre-anaerobically at an identical oxygen tension, an approximately equal percentage increase was observed over a wide range of tensions.

4. The implications of these observations and auxiliary observations dealing with the rate of the heart-beat under various conditions are discussed insofar as they shed light on the mechanism of the pre- and post-anaerobic respiration. It is concluded that diffusion alone cannot be the sole limiting factor that reduces the over-all oxygen consumption when the tension is lowered below a critical point, and the idea is discussed that the oxygen tension as such may be more important for certain cellular processes than usually assumed.

LITERATURE CITED

Alsterberg, G., 1930. Wichtige Züge in der Biologie der Süsswassergastropoden. Gleerupka, Lund.

Borden, M. A., 1931. A study of the respiration and of the function of haemoglobin in *Planorbis corneus* and *Arenicola marina. J. Marine Biol. Assoc. United Kingdom N. S.,* **17**: 709–738.

von Brand, T., 1946. Anaerobiosis in invertebrates. Biodynamica Monographs No. 4, Biodynamica, Normandy, Missouri.

von Brand, T., 1947. Physiological observations upon a larval *Eustrongylides.* XI. Influence of oxygen tension on the aerobic and post-anaerobic oxygen consumption. *Biol. Bull.,* **92**: 162–166.

von Brand, T., H. D. Baernstein and B. Mehlman, 1950. Studies on the anaerobic metabolism and the aerobic carbohydrate consumption of some fresh water snails. *Biol. Bull.,* **98**: 266–276.

von Brand, T., M. O. Nolan and E. R. Mann, 1948. Observations on the respiration of *Australorbis glabratus* and some other aquatic snails. *Biol. Bull.,* **95**: 199–213.

VON BUDDENBROCK, W., 1939. Grundriss der vergleichenden Physiologie. 2nd ed. Vol. 2. Gebruder Borntträger, Berlin.

FÜSSER, H., AND F. KRÜGER, 1951. Vergleichende Versuche zur Atmungsphysiologie von *Planorbis corneus* und *Limnaea stagnalis* (Gastropoda pulmonata). *Zeitschr. vergl. Physiol.*, **33**: 14–52.

HARNISCH, O., 1935. Versuch einer Analyse des Sauerstoffverbrauchs von *Tubifex tubifex* Müll. *Zeitschr. vergl. Physiol.*, **22**: 450–465.

HARNISCH, O., 1936. Primäre und sekundäre Oxybiose der Larve von *Chironomus thummi* (nebst ergänzenden Messungen an *Tubifex tubifex*). *Zeitschr. vergl. Physiol.*, **23**: 391–419.

HARNISCH, O., 1948. Untersuchungen zum Wesen der Erholungsatmung. *Zeitschr. Naturforschung*, **3 b**: 453–457.

HARNISCH, O., 1951. Hydrophysiologie der Tiere. Vol. 19 of: Thieneman, A., Die Binnengewässer. Schweizerbarth, Stuttgart.

JATZENKO, A. T., 1928. Die Bedeutung der Mantelhöhlenflüssigkeit in der Biologie der Süsswasserlamellibranchier. *Biol. Zentralbl.*, **48**: 1–25.

LEHMANN, J., 1935. Über den Sauerstoffverbrauch bei der vitalen Bersteinsäureoxydation in Abhängigkeit von pH und Sauerstoffdruck. *Skand. Arch. Physiol.*, **72**: 78–91.

MASSEY, V., AND W. P. ROGERS, 1951. Conditions affecting the action of fluoroacetate on the metabolism of nematode parasites and vertebrate animals. *Australian J. Sci. Research, Ser. B.*, **4**: 561–574.

MEHLMAN, B., AND T. VON BRAND, 1951. Further studies on the anaerobic metabolism of some fresh water snails. *Biol. Bull.*, **100**: 199–205.

SCHADE, A. L., AND H. LEVY, 1949. Studies on the respiration of the white potato. III. Changes in the terminal oxidase pattern of potato tissue associated with time of suspension in water. *Arch. Biochem.*, **20**: 211–219.

ZIMMERMAN, J. F., 1949. Absolute reaction rate theory and the respiratory rebound. *Biochem. Biophys. Acta*, **3**: 198–204.